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ORIGINAL CONTRIBUTION

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Investigations on the intestinal availability of native thiamin in selected foods and feedstuffs

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Summary The aim of the present study was to investigate the precaecal digestibility as a quantitative measure for the intestinal availability of naturally occuring thiamin from selected foods and feedstuffs. Therefore, three experiments were conducted to examine the following foods and feedstuffs: Eggs, bananas, white cabbage, corn, milk, fish, barley, soybeans, rice, wheat bran, brewer's yeast, rye and soybean meal. The foods and food processing procedures were made with regard to their relevance in human and animal nutrition.

For all experiments male pigs with an initial live weight between 33 and 40 kg were fitted with an end-to-end ileo-rectal anastomosis with preserved ileo-caeco-colic-valve. Three weeks after surgery, the digestibility trials were carried out from week 4 to week 9 and week 12 to week 17 after surgery. The animals were fed the individual experimental diets for a period of 12 days while digesta were collected twice a day quantitatively during the final 5 days of this period.

Precaecal digestibility for thiamin from all tested foods and feedstuffs was within a range from 73% to 94% with the highest values from boiled soybeans, boiled rice and barley, and the lowest value from steamed fish. In comparison with the animal products the plant products show on average a nearly equal precaecal digestibility for thiamin (87.3% versus 83.5%). Moreover, all tested foods and feedstuffs exhibit a relatively good intestinal availability of thiamin.

Key words Thiamin – intestinal availability – precaecal digestibility – ileo-rectal anastomosis – food

Introduction

Thiamin represents an essential nutritive substance. An adequate supply of dietary thiamin is important for the development and health of humans and animals. The daily amount of each vitamin that should be consumed is fixed in nutritional recommendations. To set up such recommendations it is necessary to know the bioavailability of the vitamins in foods and feedstuffs because mostly there is a difference between the intake, the absorption and the post-absorptive utilisation of a nutritive substance. The term "bioavailability" attempts to enclose a series of more or less successive metabolic processes as digestibility, absorption, organ uptake and release, enzymatic transformation, secretion and excretion. One important factor with regard to the bioavailability of micronutrients is the intestinal availability and absorption (1). Therefore, many experimental approaches have been made to determine the intestinal availability of B-vitamins especially of thiamin in foods and feedstuffs (e.g. 2–4).

In recent publications the in vivo determination of precaecal digestibility as a measure for the intestinal availability of native B-vitamins was introduced (5, 6). For the present investigation all animals were fitted with end-to-end (EEV) ileo rectal-anastomosis because in former studies this technique has been shown to be a valuable measure to determine the availability of thiamin in foods and feedstuffs (6). The pig was chosen as the animal model because in extensive studies the domestic pig was validated as a good experimental model for studying aspects of digestive physiology in humans and monogastric animals (e.g. 7, 8). The aim of the present investigation was to determine the intestinal availability of naturally occuring thiamin in a series of frequently consumed foods and feedstuffs. Some of the tested foods were prepared in such a way as they are usually eaten in human nutrition, therefore, the eggs, rice and soybeans were boiled and the fish was stewed before feeding to the animals.

Materials and methods

In three experiments a total of 12 (grouped as 3, 6 and 3) male growing pigs with 33 to 40 kg body weight were fitted with ileorectal-anastomosis in end-to-end technique with preserved ileo-caeco-colic valve (6, 9). The experimental design was a Latin square design, so that each animal received each food in the experiment.

Surgical procedures

The principle of fitting an ileorectal-anastomosis using the end-to-end technique is to completely separate the caecum and the colon so that the colon is disconnected from the functional gastrointestinal tract, and the distal section of the ileum and the proximal section of the rectum were anastomosed end-to-end by individual stitches. The colon remains in the belly as a blind part of the gut. The post-surgical process of excretion occurs in the normal way over the sphincter ani. To avoid caeco-colical tympania a percutaneous plastic canula is implanted as a permanent caecostomato in the left abdominal wall. The detailed description of the surgical procedures has been published elsewhere (6, 9, 10).

After surgery the pigs were returned to the metabolic cages. Before the beginning of the metabolic trials the animals received a wheat ration consisting of 620 g/kg wheat, 100 g/kg sucrose, 83 g/kg casein, 80 g/kg wheat starch, 52 g/kg of a mineral-vitamin mixture, 38 g/kg soybean oil, 19 g/kg cellulose and 7 g/kg added amino acids.

Metabolic trials

Each of the three experiments was conducted in two periods which lasted from 4th to 9th and 12th to 17th week after surgery. Between periods, a conventional wheat-based

Table 1 Composition and nutrient content of the diets

Food/ Feedstuff	Experiment	Daily amount of the tested food [g]	Daily amount of the supplementary diet [g]	Ingredients of the supplementary diet [%]								
				Casein vitamin free	Corn starch	Soybean oil	Cellulose	Sucrose	Water	Amino acid premix[1]	Mineral premix[1]	Vitamin premix[1]
Eggs, boiled	1	1700	880	—	62.2	—	13.0	14.7	—	—	9.2	0.9
White cabbage	1	3500	1158	7.8	55.2	3.4	—	12.9	—	14.1	5.9	0.7
Bananas	1	2700	750	12.0	15.4	14.6	4.2	20.0	—	23.4	9.4	1.0
Corn	1	975	525	25.8	10.4	8.0	5.8	28.6	—	6.1	13.9	1.4
Fish, stewed	2	1320	912	—	72.6	3.1	5.0	12.8	—	—	5.7	0.8
Rice, boiled	2	778[2]	722	12.5	23.0	12.5	—	20.8	—	18.3	11.9	1.0
Milk powder	2	455	925	—	44.9	—	7.2	15.0	12.0	14.5	5.6	0.8
Soybeans, boiled	2	588[2]	912	—	71.9	—	—	16.5	—	1.1	9.7	0.8
Barley	2	945	555	16.2	18.6	16.2	1.8	26.9	—	6.4	12.6	1.3
Wheat bran	2	330	1170	7.7	53.0	6.4	—	12.8	—	13.5	6.0	0.6
Brewer´s yeast	3	75	1425	6.3	58.3	2.4	3.6	10.5	—	11.9	6.5	0.5
Rye	3	1035	465	19.0	—	13.6	5.1	31.8	—	14.9	14.0	1.6
Soybean meal	3	458	1042	—	66.5	7.1	2.9	14.4	—	1.1	7.3	0.7

[1] Dispensable and indispensable amino acids, minerals and fat soluble vitamins were added individually to each diet to meet the optimum requirements (18)
[2] Air dry basis

Table 2 Thiamin content of the foods/feedstuffs and the compound diets

Food/Feedstuff or diet	Thiamin [µmol/kg DM[1]]
Eggs, boiled	12.23
Supplementary feed, eggs	—[2]
White cabbage	17.06
Supplementary feed, white cabbage	—[2]
Bananas	6.88
Supplementary feed, bananas	—[2]
Corn	18.25
Corn diet	9.58
Fish, stewed	13.50
Supplementary feed, fish	—[2]
Milk powder	9.74
Milk powder diet	3.59
Rice, boiled	15.79
Supplementary feed, rice	—[2]
Soybeans, boiled	33.34
Supplementary feed, soybeans	—[2]
Barley	21.61
Barley diet	13.00
Wheat bran	29.36
Wheat bran diet	7.48
Brewer's yeast	176.36
Brewer's yeast diet	6.92
Rye	16.42
Rye diet	11.50
Soybean meal	21.54
Soybean meal diet	4.09

[1] *DM* dry matter; [2] below the detection limit of < 0.17 µmol/kg DM

diet was offered for 14 days. Former experiments (5, 6, 10) had demonstrated that the results between these two periods are identical, i.e. no morphological or microbial alterations occur during this time. In each experiment the animals were given the test diets sequentially in a randomised order. In the first experiment the animals were fed the corn and egg diets during the first period and the banana and white cabbage diets during the second period. During the second experiment in the first period the wheat bran, milk powder and rice diets were tested, and in the second period barley, soybean and fish diets were fed. In the third experiment the animals received the rye, soybean meal and brewer's yeast diets during the first and second period. The preperiod lasted 7 days and afterwards digesta of all pigs were collected twice a day quantitatively over a period of 5 days.

In all three experiments the animals received different diets based on plant and animal foods (Table 1): fruits [bananas], salad and vegetables [white cabbage], animal products [eggs; milk powder; fish (Alaska pollack); brewer's yeast (Leiber, Osnabrück, Germany)] and plant products [corn; rice (Oryza, Hamburg, Germany); soybeans; barley; wheat bran; rye; soybean meal]. All of these foods and feedstuffs are commonly used in human and animal nutrition. Additionally, individually composed supplementary diets were given to the test products so that the test product plus the supplementary diet provided adequate amounts of nutrients, minerals, and vitamins. Only the experimental product tested contained the native thiamin while the supplementary diets were thiamin-free (Table 2). The pigs were provided daily with 1350 to 1400 g dry matter, an energy amount of 21.75 MJ ME and 225 g crude protein per animal.

Before the beginning of the experiment the white cabbage and the bananas were cut into small pieces and the eggs were boiled for 10 minutes and peeled afterwards. Subsequently, these test products were deep-frozen in vacuum-packed meal portions. On the morning of every feeding day the bananas as well as the cabbage and the eggs were thawed over the same time and temperature, respectively. Also every day before feeding the animals, the rice and the soybeans were cooked for 20 minutes with one difference: the soybeans were prepared in a steam-cooker. The deep-frozen fish was stewed for 10 minutes. Eggs, rice, soybeans, fish, white cabbage and bananas were combined with the supplementary diet and water plus 150 mL electrolyte solution [0.14 M Na (0.09 M NaCl, 0.05 M $CH_3COONa \times 3H_2O$), 4.99 mM K (4.52 mM KCl), 1.48 mM Mg (1.50 mM $MgCl_2 \times 6H_2O$) and 2.50 mM Ca (2.50 mM $CaCl_2 \times 6H_2O$] and fed in a semi-liquid consistency. The diets with all the other foods and feedstuffs were prepared in advance and also mixed with water and electrolyte solution to a pulpy consistency. Feeding time was twice a day at 7 a.m. and 3:30 p.m..

Analytical procedures and statistics

The collected faeces of each animal as well as the foods and feedstuffs were freeze-dried, mixed and homogenized for the vitamin analysis. In regard to the boiled, cooked or stewed foods thiamin analyses were performed after the heating process. Thiamin was analysed by a thiochrome method according to Rettenmaier and coworkers (11). The data of each experiment were subjected to oneway analysis of variance (SAS System). For significant F-values ($p < 0.05$) means were compared by the Student-Newman-Keuls test. All data in this test are expressed as means ± standard deviation of the single values. Significantly different means ($p < 0.05$) are marked by different superscripts.

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Results

The thiamin contents of the different foods and feedstuffs as well as the thiamin contents of the compound diets are shown in Table 2. As can be noted no thiamin content was detected in the supplementary diets. This means that this vitamin was exclusively provided by the test food. The thiamin contents in the individual diets (corn diet, milk powder diet, barley diet, wheat bran diet, brewer's yeast diet, rye diet and soybean meal diet) were provided by the proportion of the investigated food or feedstuff as a main component of these diets.

The highest thiamin content of all tested foods or feedstuffs was in brewer's yeast with 176.36 µmol/kg dry matter. The boiled soybeans contained a relatively high thiamin concentration which was two times higher than the concentration in the soybean meal, the residue after fat extraction. Besides white cabbage, corn, barley and wheat bran also supplied essential amounts of thiamin.

Tables 3–5 show the results of the digestibility trials (experiments 1, 2 and 3) with regard to the daily thiamin intake, the thiamin concentration in chyme, the daily thiamin excretion and the precaecal digestibility of thiamin in different foods and feedstuffs. The daily dietary intake of thiamin varied between 3.65 µmol from stewed fish and 17.86 µmol from boiled soybeans. The highest thiamin concentrations in chyme were found with the diets containing corn, stewed fish and rye. The precaecal digestibility of thiamin in all tested foods and feedstuffs was found to be relatively high, ranging between 73% in stewed fish and 94% in boiled soybeans, boiled rice and barley. The thiamin in the soybean meal had a precaecal digestibility of 89% and therefore was negligibly lower than that of thiamin in the boiled soybeans. When compared to the animal products the plant products showed on average a nearly equal precaecal digestibility of thiamin (87.3% versus 83.5%).

Table 3 Thiamin concentration in chyme, daily thiamin excretion and precaecal digestibility of thiamin from different foods/ feedstuffs (experiment 1)[1]

Food/feedstuff	Number of observations n	Daily thiamin intake µmol	Thiamin concentration in chyme µmol/kg DM[2]	Daily thiamin excretion µmol	Precaecal digestibility %
Eggs, boiled	3	5.29	5.49 ± 0.53[b]	0.97 ± 0.14[b]	82 ± 3
White cabbage	3	5.20	6.38 ± 0.30[b]	0.98 ± 0.14[b]	81 ± 3
Bananas	3	5.02	3.59 ± 2.13[b]	1.15 ± 0.29[b]	77 ± 6
Corn	2	13.08	12.04 ± 0.40[a]	2.51 ± 0.06[a]	81 ± 0

[1] Data are means ± SD. Means within each column were compared by Student-Newman-Keuls test, significantly different means are marked with different superscripts; [2] *DM* dry matter

Table 4 Thiamin concentration in chyme, daily thiamin excretion and precaecal digestibility of thiamin from different foods/ feedstuffs (experiment 2)[1]

Food/feedstuff	Number of observations n	Daily thiamin intake µmol	Thiamin concentration in chyme µmol/kg DM[2]	Daily thiamin excretion µmol	Precaecal digestibility %
Fish, stewed	6	3.65	10.94 ± 1.90[a]	0.98 ± 0.08[a]	73 ± 2[c]
Milk powder	6	4.62	4.06 ± 0.83[bc]	0.57 ± 0.11[b]	88 ± 2[b]
Rice, boiled	6	10.87	6.05 ± 2.36[b]	0.61 ± 0.22[b]	94 ± 2[a]
Soybeans, boiled	6	17.86	4.95 ± 2.03[bc]	1.05 ± 0.36[a]	94 ± 2[a]
Barley	6	17.80	3.72 ± 1.43[c]	1.07 ± 0.36[a]	94 ± 2[a]
Wheat bran	6	10.84	0.84 ± 0.21[c]	0.83 ± 0.26[ab]	92 ± 2[a]

[1] Data are means ± SD. Means within each column were compared by Student-Newman-Keuls test, significantly different means are marked with different superscripts; [2] *DM* dry matter

Table 5 Thiamin concentration in chyme, daily thiamin excretion and precaecal digestibility of thiamin from different foods/ feedstuffs (experiment 3)[1]

Food/feedstuff	Number of observations n	Daily thiamin intake µmol	Thiamin concentration in chyme µmol/kg DM[2]	Daily thiamin excretion µmol	Precaecal digestibility %
Brewer's yeast, dried	3	9.58	1.76 ± 0.45[b]	0.83 ± 0.18[b]	91 ± 2[a]
Rye	3	15.73	2.92 ± 0.20[a]	2.59 ± 0.19[a]	84 ± 1[b]
Soybean meal	3	5.56	0.73 ± 0.26[c]	0.63 ± 0.23[b]	89 ± 4[ab]

[1] Data are means ± SD. Means within each column were compared by Student-Newman-Keuls test, significantly different means are marked with different superscripts; [2] *DM* dry matter

Discussion

In the past when bioassays where used for the determination of the intestinal availability of B-vitamins (e.g. 12, 13) the endogenous vitamin synthesis by the intestinal flora was not excluded. This fact led to an alteration in the intestinal availability of the native B-vitamins from foods. Using in vitro assays there is also a problem with the limited extrapolation of the results to the human organism. To avoid these methodical disadvantages, in this investigation pigs were used as an animal model as they show a very similar structure of their gastrointestinal tract in comparison to the human gastrointestinal tract (7, 8). Besides, all animals were fitted with an ileo rectal-anastomosis in end-to-end technique to eliminate endogenous vitamin synthesis by their intestinal flora. The advantages of this method were confirmed in several studies (6, 10).

The absorption of low thiamin concentrations [up to 1.5 µM] occurs in the intestines by a saturable transport mechanism; high concentrations are absorbed by passive diffusion (14, 15). It can be assumed that in the present study the thiamin absorption predominantly followed the active process because considering the volumes of all digested liquids, of the foods and water consumed, the intraluminal concentrations of thiamin in the small intestine did not exceed a concentration of 1.5 µM with all tested foods, and, therefore, absorption through passive diffusion can be excluded in the present experiments. In addition, it is known that only pharmacologically high doses of thiamin are absorbed by passive diffusion (14, 15).

Studies on the intestinal availability of thiamin from foods are very limited. Roth-Maier and co-workers (6) found that the intestinal availability of thiamin in different foods (wheat, whole-grain bread, potatoes, roast pork and roast beef) ranged between 75% for whole-grain bread and 96% for roast pork. Yu and Kies have done their research on the field of thiamin availability and dietary fibre content from maize (4). They found that a low or marginal vitamin supply may be adversely affected by increased consumption of dietary fibre. In contrast to these results, in the present study a very good precaecal digestibility was found for wheat bran (92%). Another study dealt with the bioavailability of thiamin from green leafy vegetables in India (16). They found bioavailabilities of thiamin for the tested vegetables between 59% and 62%. The precaecal digestibility for white cabbage in the present investigation was 81%. Both studies cited (4, 16) were carried out with humans and thiamin was measured in urine. With this method the endogenous vitamin synthesis by the intestinal flora and the metabolic utilisation were not excluded. Thus data from those investigations and the present study are not comparable.

It is known that there are some factors controlling intestinal thiamin availability from foods but relatively little information is available regarding this topic (for review see 17). Generally the digestion and absorption of micronutrients like vitamins are influenced by many exogenous and endogenous factors as for example the physical and physiological status of the organism or the composition of the diet. A further study already indicated that the composition of foods influences the ileal digestibilities of the vitamins B_1, B_2 and B_6 (6). In the present investigation the precaecal digestibility of thiamin from all tested foods ranges between 73% and 94%. The highest percentages are found for boiled soybeans, boiled rice and barley while stewed fish showed the lowest precaecal digestibility of thiamin from the tested foods. The precaecal digestibilities found in this study for corn, wheat bran and milk are nearly similar to that from another study with end-to-side anastomosis (5). When compared to the animal products the plant products show similar precaecal digestibilities of thiamin (87.3% versus 83.5%). Moreover, all tested foods and feedstuffs exhibit a relatively good intestinal availability of thiamin, presumably given by its occurrence in free and phosphorylated form in these products. The relatively low intestinal availability of fish requires further research.

Based on the results of the present investigation it can be concluded that the ileorectal-anastomosis in the end-to-end technique is a suitable method to measure the intestinal availability of B-vitamins. By using of this method all tested foods and feedstuffs show relatively high intestinal availabilities of thiamin.

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